Technical Report No. IRL 1027

CYTOCHEMICAL STUDIES OF PLANETARY MICROORGANISMS EXPLORATIONS IN EXOBIOLOGY

Status Report Covering Period April 1, 1964 to September 1, 1965

For

National Aeronautics and Space Administration

Grant NsG 81-60

Instrumentation Research Laboratory, Department of Genetics
Stanford University School of Medicine
Palo Alto, California

Report to the National Aeronautics and Space Administration

"Cytochemical Studies of Planetary Microorganisms - Explorations in Exobiology"

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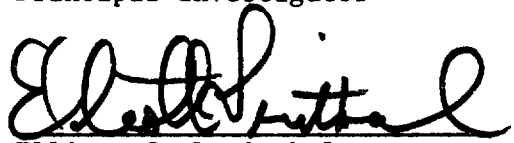


Elliott C. Levinthal
Project Director

TABLE OF CONTENTS

A. Introduction

B. Program Resume

I Multivator

II Fluorometry

III Gas Chromatography and Optical Resolution of Amino Acids

IV Mass Spectrometry

V LINC Evaluation Program

VI UV Microspectrometry

VII Selective Membrane Permeability

VIII NMR Biological Applications

IX Carbon Metabolism

C. Summary Listing of Papers and Reports

D. Personnel and Organization

INTRODUCTION

This status report covers the period April 1, 1964 to September 1, 1965. The major technical efforts have been described in separate technical reports and papers. The status report refers to these and summarizes continuing projects.

The efforts of the laboratory under this program relate to the following functions:

1. Development of specific, mission-oriented experiments for planetary exploration - e.g. Multivator and the Pasteur Probe.

2. Studies of analytical systems that have special potential in long-range plans for an automated laboratory for Mars - e.g. Mass Spectrometry and Phosphorimetry.

3. Automation of laboratory science under computer control - e.g. LINC computer programming systems; data acquisitions and reduction and closed loop instrument control; mechanized inference from experimental data (artificial intelligence).

Most of the projects summarized in this report partake of more than one of these functions to varying degrees.

Besides the Instrumentation Research Laboratory, which functions as part of the Genetics Department, the participating group includes Professors Carl Djerassi (Chemistry Department), Lubert Stryer (Biochemistry), Peter Bulkeley (Director Design Division, Mechanical Engineering), and John McCarthy and Edward Feigenbaum (Computer Sciences), and their associates.

Work under this grant includes areas of research that are closely related to efforts being carried out in the Department of Genetics under other grants or contracts. This includes Air Force Contract AF 49(638)1599 For "Molecular Biology Applications of Mass Spectroscopy", National Institute of Neurological Diseases and Blindness Grant NB-04270 entitled "Molecular Neurobiology" and a MACY Foundation Grant for "Planning for Advanced Computer Facility - The Stanford Medical School". Work under this latter grant is preparatory to carrying out the Advanced Computer for Medical Research (ACME) proposal submitted to National Institute of Health, Division of Research Facilities and Resources. In addition, there is collaboration with the work in the Computer Science Department on artificial intelligence carried out under support of the Advanced Research Projects Agency SD 183. The relationship of the work carried out under the NASA grant to these other activities has proved of great mutual benefit in all cases.

The general project areas of the Resume are:

I	Multivator
II	Fluorometry
III	Gas Chromatograph and Optical Resolution of Amino Acids
IV	Mass Spectrometry
V	LINC Evaluation Program
VI	UV Microspectrometry
VII	Selective Membrane Permeability
VIII	NMR Biological Application
IX	Carbon Metabolism

A summary listing of all reports and papers published under the grant is included. This does not include the many papers presented to various academic and professional groups by members of the staff. Information covering the present organization and staff of the IRL is also presented.

PROGRAM RESUME

I Multivator. The general concept of multivator as an instrument has been described previously. The mechanical design work has been carried out by the Design Division of the Department of Mechanical Engineering in close cooperation with the Instrumentation Research Laboratory of the Genetics Department. This work has been included in a report submitted to NASA under this grant, entitled "Multivator Design Studies", September 1965.

The initial concept of multivator was based on carrying out single step chemical reactions. Several design studies were carried out based on this concept. A pneumatically actuated unit was designed for field-test purposes (Figure 1 & 2). Each of its three modules could be easily disassembled, sterilized, and reloaded with solvents and substrates. The moldules used a compressed air supply for actuation reducing the cost of field-tests in contrast to the systems design which relied on squibs for actuation. Only two moving parts were used in each module. Both are pistons, one used for valving, and the other for injection of the solvent. A dynamic analysis of this device is given in the above referenced report.

Work on a mechanical design of single step multivator systems has been carried to a point which would permit one to fairly rapidly arrive at final design specification, should a mission requirement arise for such a device. The limitations of single step chemical reactions are discussed more thoroughly in the section of this report under fluorometry; work on the single step design concept has been terminated.

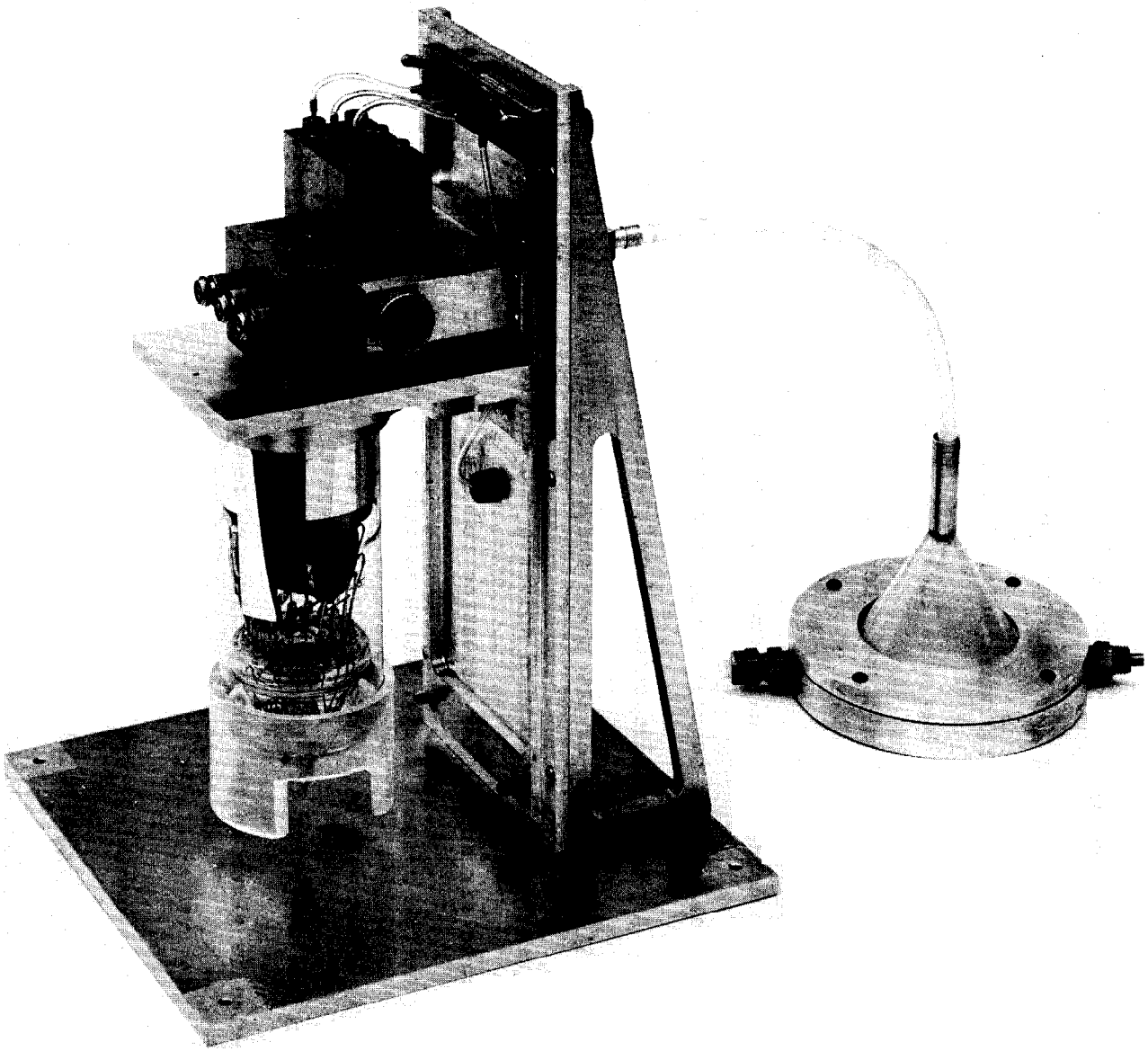


Figure 1. Field-Test Multivator

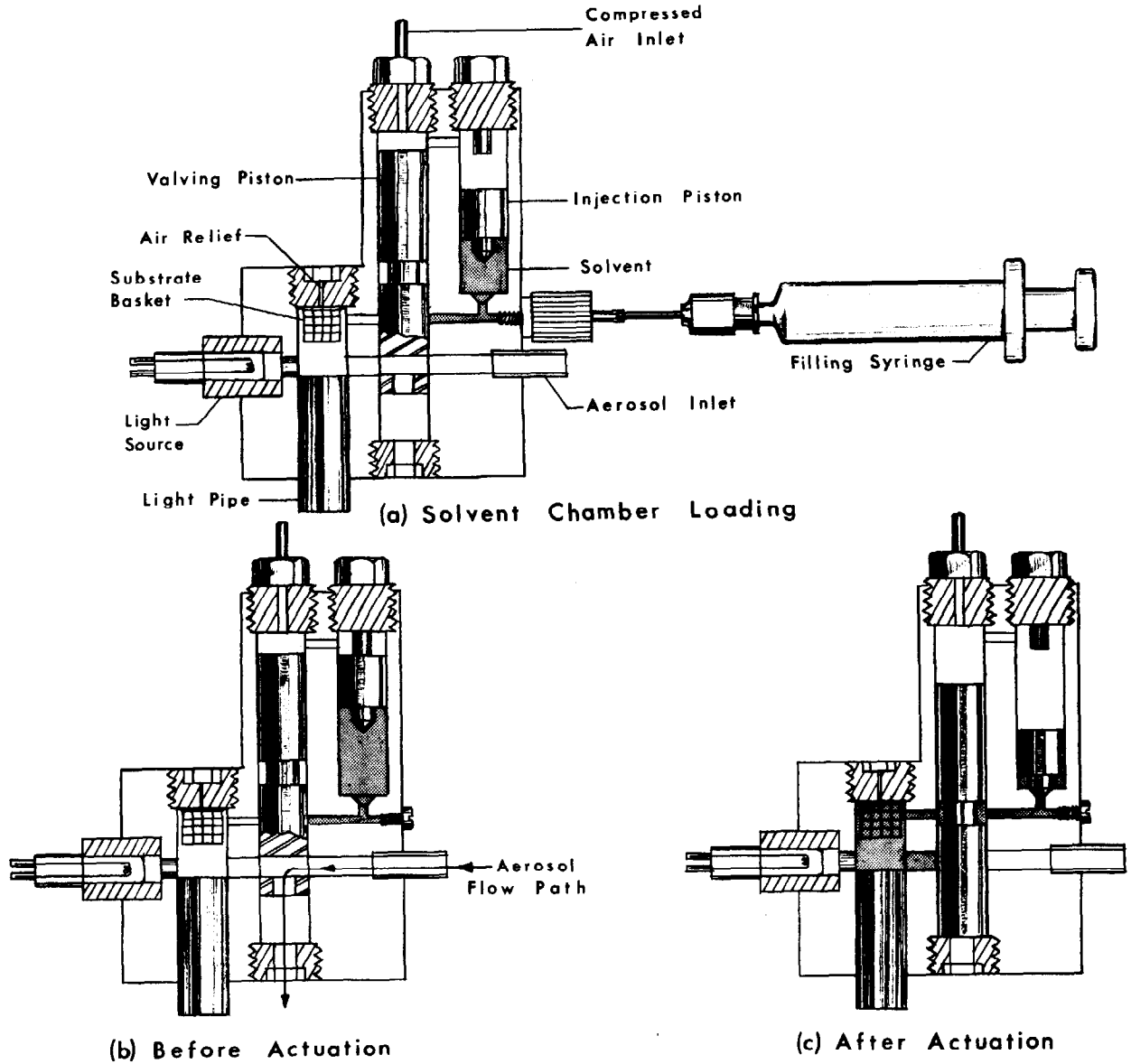


Figure 2. Field-Test Multivator Operational Diagram

A laboratory test unit (Figure 3) was designed to study the problems of carrying out a complex sequence of metering, mixing, dilution and filtering operations associated with multi-step chemical processing. The design of this unit is based on a recently developed heat sterilizable substrate used for the phosphatase assay (See Section II). The substrate is initially stored in the form of two separate components which are individually heat sterilizable. The substrate components are mixed immediately prior to use to avoid chemical breakdown during sterilization and subsequent storage.

A flow diagram (Figure 4) indicates the number of operations carried out by the test unit. The unit provides the three samples normally used for hydrolytic enzyme analysis; one for assay, the other two for control. It does not provide, however, for the photometric assay itself.

The laboratory makes use of pneumatic actuators for expelling the contents of glass syringes, and motor-actuated valves for directing the flow through the system. The sequence control of the unit is carried out by the LINC computer. This unit will be operationally tested shortly.

In addition to the work referred to above, other mechanical design work has been carried out that is pertinent to multivator and related instruments. A comprehensive study was made of soil sampling problems. Various soil collecting and processing techniques were explored. The results of this study are contained in "Multivator Design Studies". A sample collector (shown in Figure 1 of this report) was designed for use with the multivator field test-unit. The collector is selective of particles up to 100 microns in diameter and has

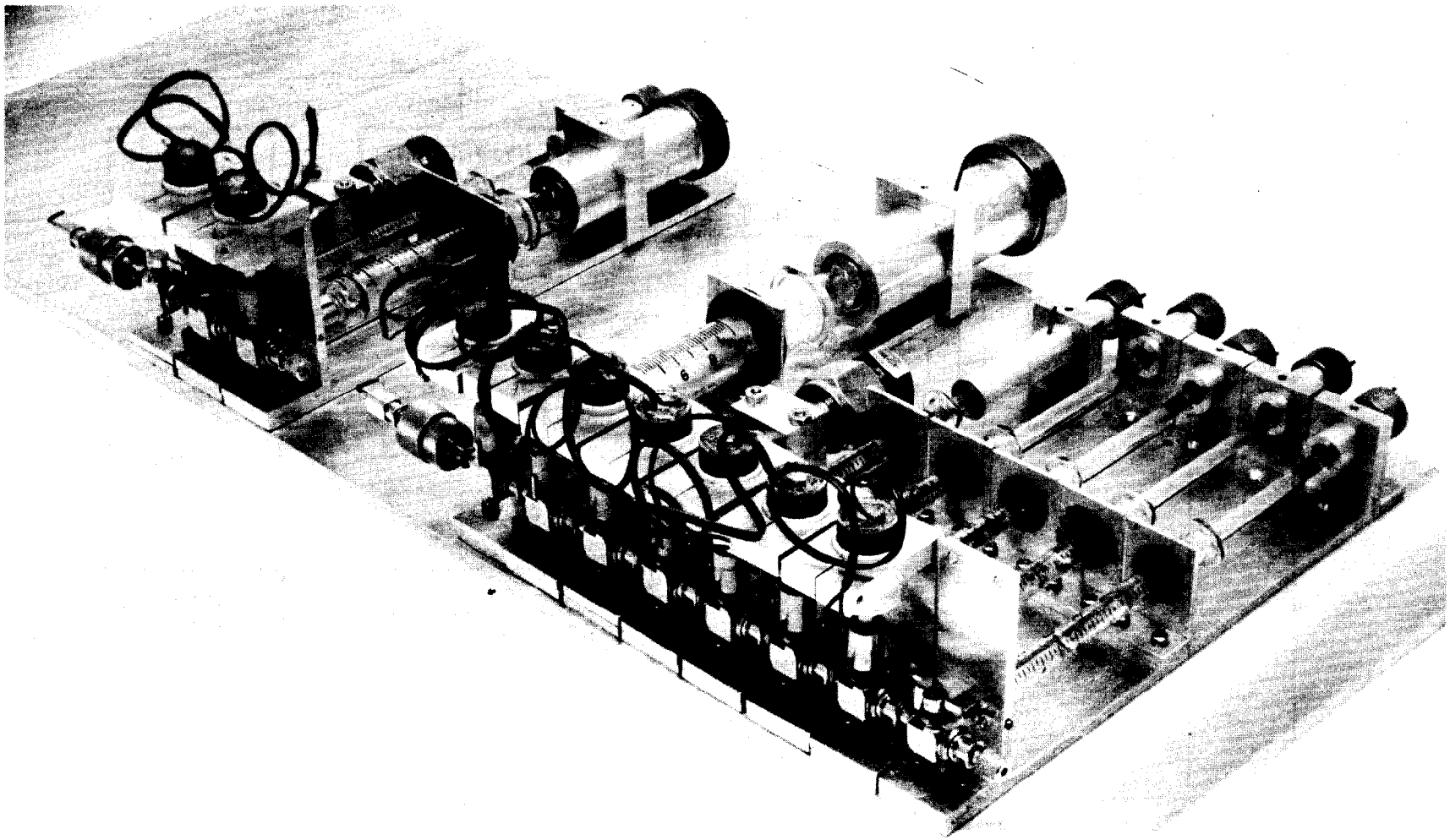
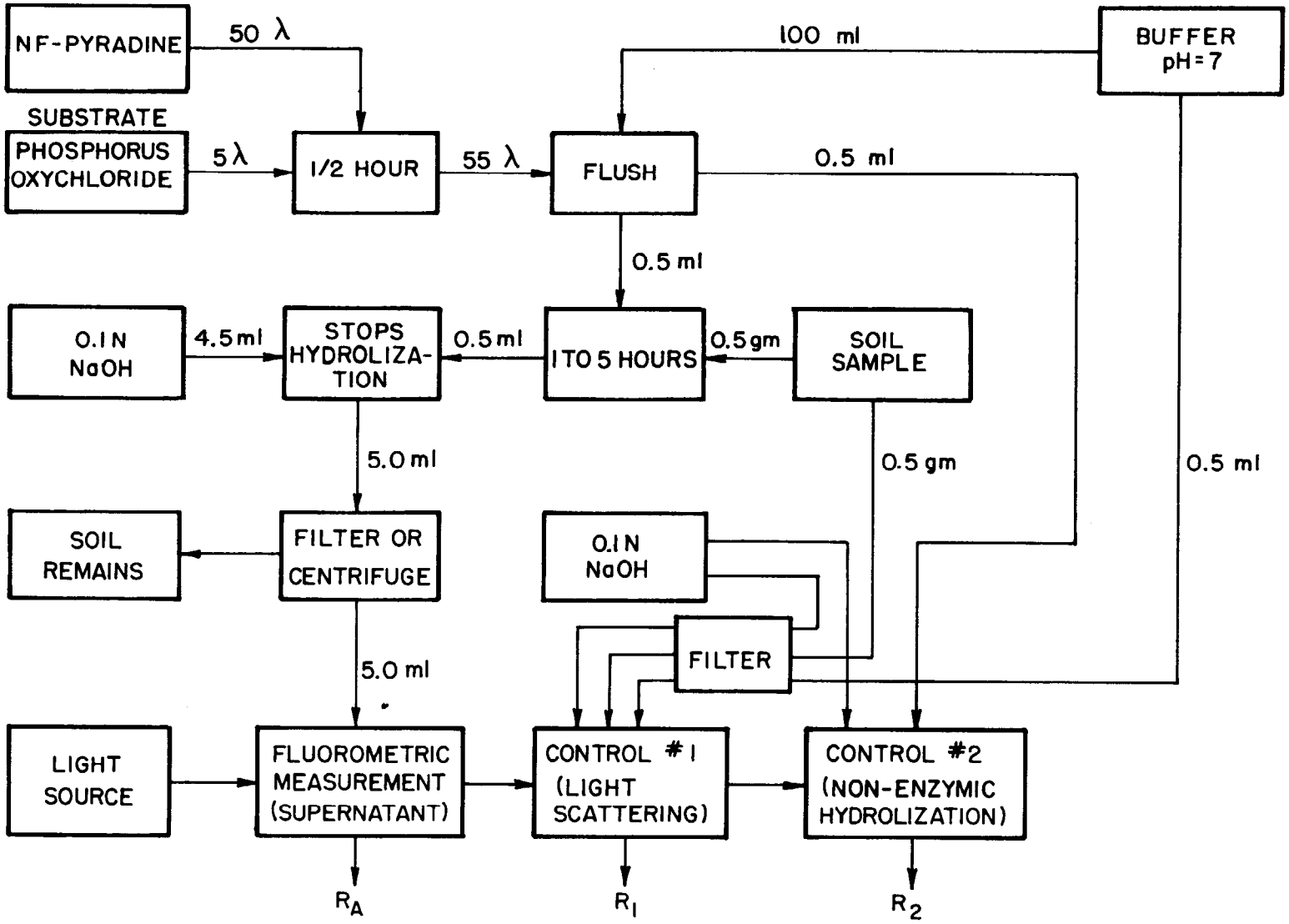


Figure 3. Wet Chemistry Laboratory



READING = $R_A - R_1 - R_2$

Figure 4. Wet Chemistry Laboratory Flow and Process Schematic.

been tested with a wide variety of particles including gravel, dirt, chopped leaves, and grated glass beads. The collector is described in more detail in the same report.

Two studies were conducted to determine the most appropriate type of mechanism actuators for use in multivator systems. Performance measurements were made on explosive squibs of a type used in the squib actuator multivator. Such characteristics as energy and force output, and dynamic response were determined. Comparisons were made of a bellows motor to chemical and mechanical energy sources. A study was also made of solenoid actuators taking into consideration such factors as work-to-size ratio, and duty cycle. In connection with the design of the multi-step processing unit, the problem of metering small quantities of liquids was studied. The results of these three studies are also described in the Mechanical Design Division report referred to above.

Optical design studies were carried out relating to miniaturized fluorometric assays. The problem study related mainly to the light source and optical systems associated with them. Ideally, the active area of the excitation source should be comparable with the dimensions of the circle of confusion of the collimating lense, a condition which will be difficult to approach with known light sources when space is at a premium. Although commercially available miniature tungsten light sources depart grossly from the ideal noted, some practical experiments were undertaken to evaluate their performance. The test unit was set up in as simple a form as possible and was intended to simulate a part of a typical multivator design.

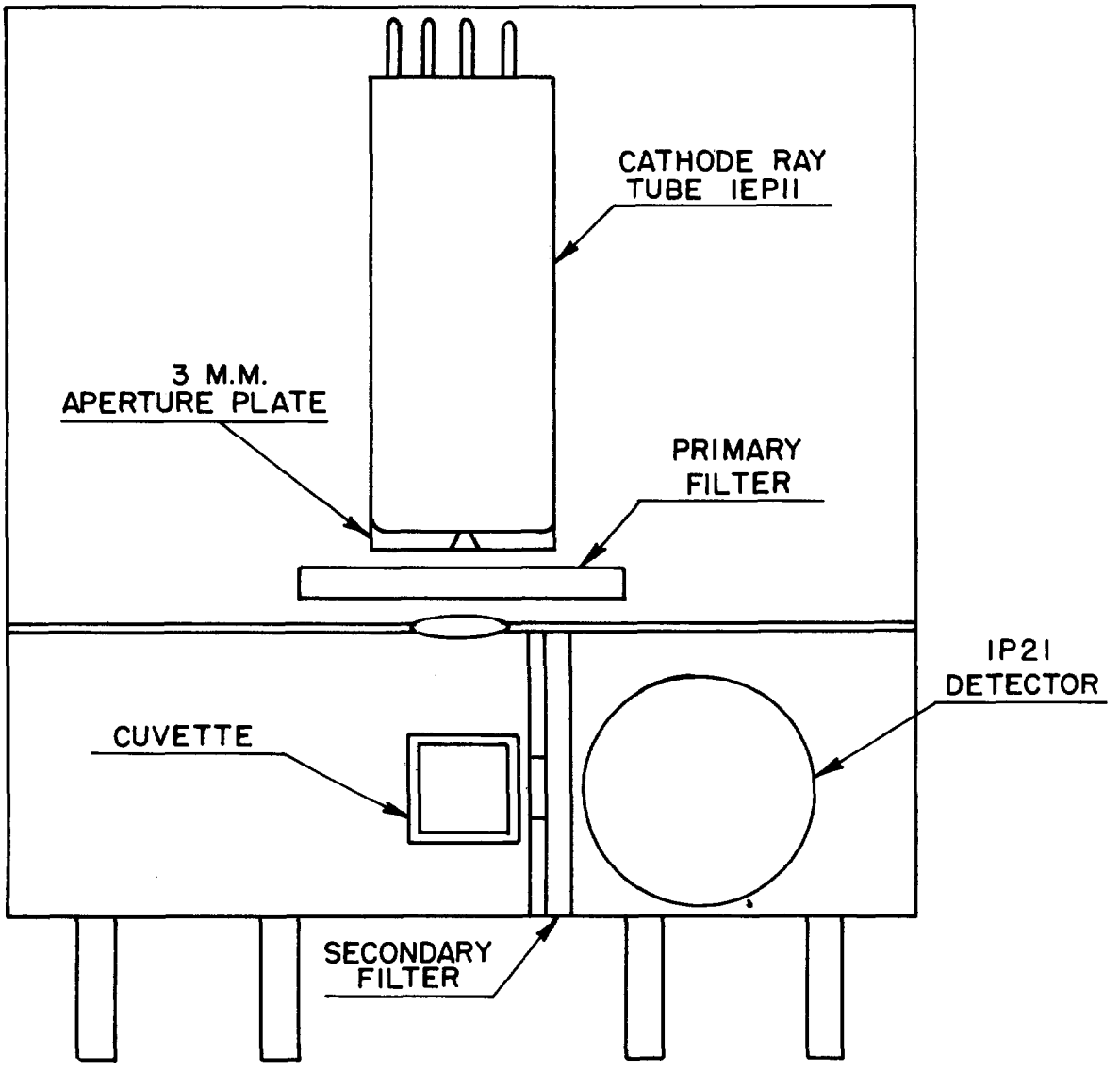


Figure 5. Fluorometry Test Unit Mark II

An aluminum block was machined to provide two chambers of 0.2 milliliters capacity. These chambers could be alternately illuminated via a Wratten 47B filter by miniature tungsten lamps operated at 5V, 80ma. The bottom of the chambers were sealed by polished lucite plugs which also served to direct the fluorescent output to the photo-cathode of an R.C.A. type 6199 multiplier phototube via a secondary filter, Wratten 52. The output of the multiplier tube amplified and displayed on a Tektronix oscilloscope was used in this instance as a measuring device.

One chamber was filled with distilled H_2O, the other with various dilutions of fluorescein. It was found that excessive scattering of the excitation light by the chamber and bulb aperture walls limited the utility of the system. Concentration of fluorescein of 10^{-6}M solution and greater only could be differentiated from the H_2O. An attempt was made to improve the primary filtration by adding another Wratten 42B filter, but the resulting degradation in signal to noise ratio resulting from an inadequate light source precluded meaningful measurements.

An alternative system was fabricated (See Figure 5) which permitted the use of a standard rectangular cuvette, and in which primary and secondary filters could be changed as required. A simple lens of 12mm. focal length and approximately 0.4 N.A. served as a collimator. An R.C.A. 1P21 multiplier phototube was used as a detector. Provision was made for the use of either a tungsten lamp or a miniature cathode ray tube, R.C.A. type 1EP11, as excitation sources.

In one experiment the tungsten lamp operated at 5V, 80ma. was switched on and

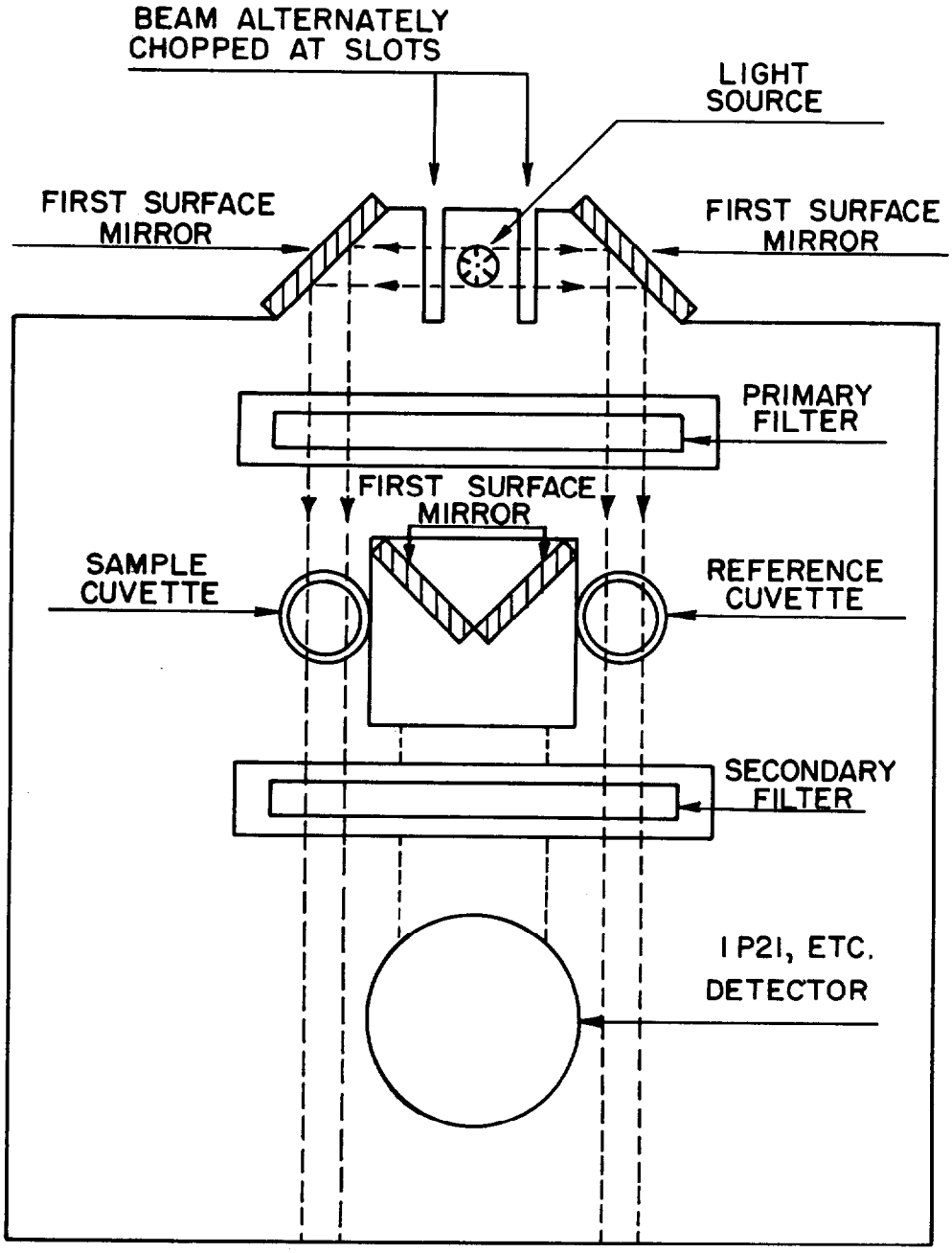


Figure 6. Flurometry Test Unit Mark III

off at four cycles per second under the control of the LINC computer. The amplified output of the detector was then processed by the computer to obtain a signal which had been averaged for twenty seconds. A dilution of 10^{-11}M fluorescein was measured for eight consecutive periods, and an equal volume of distilled H_2O for 19 consecutive periods. The following readings were obtained with primary filter Wratten 47B, secondary Wratten 52.

FLUORESCEIN 10^{-11}M	DISTILLED H_2O
5.105, millivolts	3.540, millivolts
5.046, "	3,587, "
5.085, "	3.507, "
5.066, "	3.550, "
4.983, "	3.519, "
5.101, "	3.595, "
4.944, "	3.497, "
4.905 "	3.540, "
	3.486, "
	3.558, "
	3.605, "
	3.569, "
	3.529, "
	3.470, "
	3.566, "
	3.540, "
	3.431, "
	3.456, "
	3.530, "

The median value obtained for the 10^{-11}M fluorescein was 5.027 millivolts, and that for distilled H_2O 3.350 millivolts. Since the signal noise ratio was nearly 75:1 it appears that dilutions of fluorescein approaching 10^{-13}M could be measured.

In a second experiment the tungsten lamp was replaced by the 2.54 cm. diameter cathode ray tube. The phosphor in the tube has the following relative spectral energy distribution:

4000°A - 10%
4300° - 50%
4600° - 100%
5100° - 50%
5500°A - 10%

It is thus well suited to the excitation of fluorescein. The defocussed beam of this tube, at an average current of 400 micro-amps was swept past a fixed aperture of 3mm diameter, the aperture then acting as a pulsed light source. The remainder of the optical train remained as in the previous experiment. The output of the detector, appearing as voltage pulses across a 100K resistive load, was amplified by a Fairchild ADO 3 operational amplifier arranged in a parallel tee configuration. The gain of the amplifier in this mode was 2000, and the bandwidth of the order of one cycle per second. The amplifier output was measured by a Hewlett Packard 400H V.T.V.M. and the following readings obtained:

Amplified noise and stray pick-up - 3 millivolts

0.01M Na OH	14 "
10^{-11}M Fluorescein	16 "
10^{-10}M Fluorescein	21.5 "
10^{-9}M Fluorescein	82.0 "
10^{-8}M Fluorescein	47.0 "

The limit of the Turner fluorometer, used as a reference in the two experiments described, is approximately 2.5 X 10^{-11}M for fluorescein. The results obtained indicate a comparable sensitivity.

This system was then tested under conditions that would be applicable to the use of 6,6' - dihydroxy-napthofluoran (NF) as a potential assay substrate.

The use of this substrate has great advantages with respect to its stability. These are discussed in a subsequent section of this report. However, it presents several problems from the point of view of instrument design. The quantum yield has been found to be approximately 0.05 compared to the near unity yield of fluorescein. The relative closeness of excitation and emission peaks in the visible spectrum places stringent requirements upon the filter system. The peak wavelength of fluorescent output occurs in a region poorly covered by detectors.

Using instrumentation similar to that described above, some measurements were made of NF in various dilutions. In one set of measurements where a tungsten lamp modulated at ten cycles per second was the excitation source, a 5950 Å interference filter the primary and Corning CS 2-58 the secondary filter, the following results were obtained:

0.01M NaOH	1.5 ± 0.5 millivolts
10^{-8}M NF	1.5 ± 0.5 "
10^{-7}M NF	2.6 ± 0.5 "
10^{-6}M NF	16 ± 0.5 "

The averaging period of the amplifier was approximately ten seconds. The absorption curve of NF shows a minor peak between 4000 Å and 4700 Å. When a Wratten 47B filter was substituted for the primary interference filter the figures obtained were:

0.01 NaOH	0.7 ± 0.25 millivolts
10^{-8} NF	0.8 ± 0.25 "
10^{-7} NF	1 ± 0.25 "
10^{-6} NF	1.4 ± 0.25 "

The detector used in both latter sets of measurements was an R.C.A. 7102. This phototube has peak response at about 8000 Å and was selected at a time when it was first known that a red emitting fluor was being considered for use in assays. Subsequently available data indicates that a more favorable signal to noise ratio might be obtained by the use of a detector with S-20 response.

The unacceptably low levels of detection of NF indicated the need to systematically explore the wide range of potentially usable filters and light sources and to improve the stability of the measurements. An improved system has been devised and partially constructed to meet this need. (See Figure 6.) A rotating mechanical chopper driven by a synchronous motor alternately opens and closes two light paths so that a sample and a reference cuvette receive equal excitation from a common source. Fluorescence and scatter information is detected via a mirror pair at a photocathode common to both light paths. Although a miniature tungsten bulb will be used initially, to relate results to others described, provision is made to employ virtually any other light source by use of a light guide.

II Fluorometry

A. Reagents for Fluorometric Assays of Hydrolitic Enzymes.

The studies on fluorescein phosphate and dihydrofluorescein phosphate have been completed and are covered in reports already submitted. (IRL report #1010 and IRL #1015). Work is continuing on napthofluorescein phosphate and its synthesis in situ from heat stable reagents. A paper by J.W. Westley describing the work already completed has been published [paper 26 (1965)].

Dr. V. Stevens of the Department of Chemistry of Eastern Washington State College has carried out work in our laboratory to investigate the possibility of preparing a substrate for dioxyribonucleic acid assay by means of a fluorescent technique. The dye is bound to nucleic acid so that with nuclease action the dye is either released and fluoresces strongly, or is released in such a way that it can penetrate a membrane and its fluorescence measured. The latter situation would not require that the dye be non-fluorescent while bound. Work on this project has been carried to the point of demonstrating its suitability, and is presently not continuing. The efforts to date are covered in IRL report #1029.

In cooperation with Professor Lubert Stryer of the Department of Biochemistry, Stanford University, work is progressing on the development of a fluorogenic substrate that could function for a broad array of enzymes. The method depends upon the fact that the fluorescence of a group F can be quenched by a transfer over distances as large as 30Å to an appropriate quencher Q. If F and Q are separated by a series of chemical bonds,

F-B-B-B-B-Q, then F will be non-fluorescent until one of the susceptible bonds B is attacked by an enzyme. Work on this project is continuing.

Again in cooperation with Professor Stryer, the Instrumentation Research Laboratory developed a spectrofluorometer using ratio amplification for the direct recording of corrected excitation spectra and fluorescence polarization spectra. This spectra fluorometer was used in work on fluorescent probes of protein structure. The work is continuing. The work to date is described in a recent publication [paper 24 (1965)].

B. Nanosecond Flash Fluorometry (Phosphorimetry).

A Fluorometer has been developed which is capable of following the kinetics of fluorescence in a nanosecond time range. This instrument enhances the scope of fluorescence methods in the following way:

1. It will increase the resolution of fluorescence techniques and the analysis of fluorescence molecules. Molecules with similar emission spectra can be distinguished on the basis of differing excited state lifetimes.

2. Energy transfer can be detected and studied by kinetic criteria.

3. The rotational relaxation time of fluorescence groups can be obtained directly and explicitly from a knowledge of the kinetics of the parallel and perpendicularly polarized components of the emission.

This instrument is now completed and being applied by Professor Stryer for studies along these lines. A report on the instrument will be prepared.

III Gas Chromatography and Optical Resolution of Amino Acids.

A submitted report entitled, "A Pasteur Probe, A Proposed Experiment", outlines the basis of this laboratory's interest in this problem. It is based

on the well-known significance of optical activity as a clue for the recognition of life. It provides a method whereby important metabolites like amino acid can be scanned for optical activity with very high sensitivity. Some aspects of the development of this technique are reported in several published papers [papers 14-19 (1965)]. These studies are continuing, using the same approach that has been successful for amino acids, in an attempt to generalize the application of the method to other optically active species.

To provide unequivocal identification of peaks, the gas chromatograph is being coupled to a mass spectrometer. This is initially being done, utilizing conventional techniques, by employing a Bendix Time-of-Flight Mass Spectrometer. Also under development in cooperation with the Design Division of the Stanford Mechanical Engineering Department is a new mass spectrometer inlet system which will permit the continuous introduction of solid samples into a mass spectrometer. This inlet system, together with freezing of the effluent from the gas chromatograph, will permit full advantage to be taken of the potential sensitivity of the combination of mass spectroscopy and gas chromatography.

IV Mass Spectrometry.

The techniques of mass spectrometry are presently a major concern of the Instrumentation Research Laboratory.

A. Analysis of Natural Products.

The Atlas CH-4 Mass Spectrometer has been installed in Professor Djerassi's

laboratory in the Department of Chemistry. The work there on the analysis of natural products has yielded the results reported in the following papers:

Aplin, R. T.; Budzikiewicz, H; Horn, S.; and Lederberg, J.: Logarithmic Recording of Mass Spectra, Especially Peaks from Metastable Ions. Anal. Chem., 37, 776 (1965).

Walser, A.; and Djerassi, C.: Alkaloid Studies LII. The Alkaloids of Vallesia Dichotoma. Helv. Chim. Acta, 48, 391 (1965).

Budzikiewicz, H.; Brauman, J. I.; and Djerassi, C.: Mass Spectrometry in Structural and Stereochemical Problems. LXVII. Retro-Diels-Alder Reaction of Organic Molecules Under Electron Impact. Tetrahedron, 21, 1855 (1965).

Duffield, A. M.; Budzikiewicz, H; and Djerassi, C.: Mass Spectrometry in Structural and Stereochemical Problems. LXX. A Study of the Fragmentation Processes of Some Five-Membered N-Alkyl Lactams and N-Alkyl Succinimides. J. Am. Chem. Soc., 87, 2913 (1965).

Duffield, A., Budzikiewicz; and Djerassi, C.: Mass Spectrometry in Structural and Stereochemical Problems. LXXI. A Study of the Influence of Different Heteroatoms on the Mass Spectometric Fragmentation of Five-Membered Heterocycles. J. Am. Chem. Soc., 87, 2920 (1965).

Aplin, R. T.; Budzikiewicz, H; and Djerassi, C.: Mass Spectrometry in Structural and Stereochemical Problems. LXXIII. The Negative Ion Mass Spectra of Some Simple Organic Compounds. J. Am. Chem. Soc., 87, 3180 (1965).

Jackson, A. H.; Kenner, G. W.; and Smith, K.M.; Aplin, R. T.: Budzikiewicz, H.; and Djerassi, C.: Mass Spectrometry in Structural and Stereochemical Problems. LXXVI. Pyrooles and Related Compounds. VIII. The Mass Spectra of Porphyrins. J. Chem. Soc., in press.

B. Data Acquisition.

The Instrumentation Research Laboratory developed a special logarithmic amplifier for use with the Atlas Mass Spectrometer. This development is described in the report entitled, "The Use of the Logarithmic Amplifier and Data Processing of Analog Signals". (IRL report #1017). A second similar log amplifier was built for the Bendix Time-of-Flight Mass Spectrometer, incorporating a null or base line setting circuit. Preliminary investigation of data acquisition and analysis problems have been carried out using an analog tape recorder to initially acquire the data. The LINC computer is then used for A to D conversion and to serve as a data processor to produce a format useable by the B5500 or IBM 7090 computer. The processed data is then stored utilizing a digital tape recorder. The analog tape interface will be eliminated by direct connection of the mass spectrometer to the A to D conversion system. Provision is also being made to eliminate the need for the digital tape recorder by providing a direct digital link from the LINC Computer to the B5500 or IBM 7090 computers at the Computation Center.

C. Mass Spectrometry Image Scanning.

A research program has begun which is directed toward the development of methods of microidentification and physical analysis of the cellular and subcellular constituents of biological structures. Mass spectrometric methods of surface microanalysis are being pursued. A project is underway to study the nature of the ionized fragments ejected from biological targets subjected to inert gas ion bombardment. A replica of a prototype unit developed by G. Slodzian and R. Castaing, consisting of an inert gas ionizer, primary ion gun

target support assembly, and secondary ion electrostatic lens structure has been purchased. The nature of the secondary ions produced by the primary ion bombardment of targets of interest is to be determined by use of a 60° single focusing mass spectrometer that has been designed and constructed for the purpose of this project. Initial tests are now underway.

D. Mass Spectrometry Data Interpretation.

Work on this aspect of the automation of mass spectroscopy is continuing. Work to date is contained in three publications by Professor Lederberg entitled, Computation of Molecular Formulas for Mass Spectromety; "Tables and Algorithms for Calculating Functional Groups of Organic Molecules" and High Resolution Mass Spectrometry"; and "A Subalgol Program for Calculation of Composiontal Formulas From Mass Spectra Data". In addition, a study carried out by Professor David Luenberger of the Stanford Electronics Laboratory was submitted on "Resolution of Mass Spectrometer Data".

While the high resolution mass spectrometer is perhaps the most capable single instrument for organic structural analysis, the sheer volume of its signal output poses formidable problems of data reduction and data analysis. At one level, the problem is the identification of mass numbers with compositional formulas. However, no mass spectral signal is free of noise and great effort must then be spent to obtain an accurate determination of mass to ultimate resolution. Much of this effort is wasted when it does not answer a concrete question, i.e., which of a set of possible compositions is indicated by a given measurement. Even for all compositions, the corresponding mass

numbers are not continuously distributed; they are rather the discrete set of numbers calculated from linear integral sums of nuclidic masses, and represented in the tables.

The tabulations and calculation programs just listed are the first step in a control program for the mass spectrometer. As soon as the peak is identified within a given mass neighborhood, the competing possibilities should be computed, then weighted in accordance with any other available information. This allows the experimental problem to be restated as a choice among competing possibilities, and the signal information need be accumulated only long enough to lead to a meaningful choice among them.

The same consideration applies to the telemetry of mass spectral data. The transmission of the complete spectrum as analog output might require up to a million bits per spectrum. Before telemetry, the spectrum should be analyzed as that sum of a limited number of apparent digitized components which gives a satisfactory fit, leading to some few hundred bits for a complete spectrum and a few tens of bits for the salient features.

The chemist faces the same problem in reading the data, and should have analogous computational aid. These considerations would, of course, be multiplied by the number of samples that would be processed by the micro-scanning mass spectrometer we are developing.

In solving a structure, the chemist hypothesizes a series of trial structures, then matches them with the data (in this case a mass spectrum, but this can

be generalized to any data set) and accepts or rejects his trial solutions, usually part by part, in a structure. Much of this tedious effort could be emulated or at least assisted by the computer in a program we call "mechanized induction".

For this purpose, a language which has been devised for representing chemical structures in easily computable form; "Dendral '64" [report 1 and paper 22 (1965)] . The development of this language required the filling of a surprising gap; the systematic application of simple topological principles to the field of chemical graphs - that is, a symbolic representation of organic molecules. Existing notations were found to be quite defective as the chemist already knows too well from his difficulties with nomenclature (other organizations like Chemical Abstract Service also recognize the problem and are working on it, but tend to compromise topological rigor for the benefit of established traditions in notation). At any rate, with the help of some theorems on canonical forms of trees, and on Hamilton circuits of planar maps (for acyclic and cylic structures respectively), a complete system has been worked out. This gives an algorithm by which the computer can generate an exact list of all isomers in a given composition.

By itself this is a futile approach to any but the simpliest problems, since the number of possible isomers quickly exceeds the range of a fast computer. Heuristic and symbiotic methods are therefore called for whereby the computer emulates or cooperates in the use of human problem solving techniques in searching wisely selected parts of the space of possible solutions.

Professor Edward Feigenbaum and Dr. Richard Watson of the Computer Science Department are leading a cooperative effort to program efficient displays of structural ideas for conversational interaction with the computer. This is a step to evaluate the chemists problem solving heuristics incorporating them in the machine program. The effort is limited at present by the existing computer facilities (a PDP-1 machine) with inadequate displays. These badly need augmentation for this type of work and for this purpose we are looking forward to the next generation systems planned for 1966. Meanwhile, most of the components of the Dendral 64 language have been coded as running subroutines. The system is then far enough along 1) to assure its realizability in a comprehensive program and 2) to be applicable to any special problems that might arise with the help of some ad hoc patching where general purpose interfaces have still to be built. Since this aspect of computer programming can sometimes take even much longer than working out the basic ideas, our policy is to defer this until we are operating on the next generation computer system.

In a preliminary study carried out to provide insight into operational problems for computer control, a comprehensive set of spectra of amino acids was collected, as observed with solid samples in the Bendix Time-of-Flight instrument. This work was jointly supported by this grant and National Institute of Neurological Diseases and Blindness Grant NB-04270, and Air Force Grant AF-AFOSA-886-65, and was submitted to NASA in IRL Report 1035.

V. LINC Evaluation Program

The major effort under this program has been completed and is described in the report "LINC Evaluation Program" submitted earlier (IRL #1023). We place special emphasis on the successful development of an operating system for this small computer, which is indispensible to its role as a versatile data-acquisition device. The LINC is clearly inadequate to deal directly with the more sophisticated programs that have just been discussed (Section IV D) and in any case, essentially lacking time sharing capability, it can only be dedicated to one instrument at a time. It is expected, however, to play an important role in future more elaborate systems as a peripheral computer, acting as a reasonably high speed buffer in maintaining control of and acquiring data from certain instruments which require continuous interaction. In some cases, the assignment of a secure priority level to the interaction of such an instrument, with a more sophisticated computer and time sharing system, could be expected to lead to irremediable conflicts. While it may eventually be possible to resolve these problems, given a sufficiently capable computer, the most prudent course for the near future is to maintain the special benefits - both of small peripheral computers and the large central time shared system. To a large extent the problem is the uncertainty of the demands that different peripheral devices will be placing on the central system and these remarks should therefore not be taken as a predicate for the design of an eventual integrated computer managed laboratory system.

VI. UV Microspectrophotometry.

Our efforts on video scanning techniques were originally applied to the

Professor Edward Feigenbaum and Dr. Richard Watson of the Computer Science Department are leading a cooperative effort to program efficient displays of structural ideas for conversational interaction with the computer. This is a step to evaluate the chemists problem solving heuristics incorporating them in the machine program. The effort is limited at present by the existing computer facilities (a PDP-1 machine) with inadequate displays. These badly need augmentation for this type of work and for this purpose we are looking forward to the next generation systems planned for 1966. Meanwhile, most of the components of the Dendral 64 language have been coded as running subroutines. The system is then far enough along 1) to assure its realizability in a comprehensive program and 2) to be applicable to any special problems that might arise with the help of some ad hoc patching where general purpose interfaces have still to be built. Since this aspect of computer programming can sometimes take even much longer than working out the basic ideas, our policy is to defer this until we are operating on the next generation computer system.

In a preliminary study carried out to provide insight into operational problems for computer control, a comprehensive set of spectra of amino acids was collected, as observed with solid samples in the Bendix Time-of-Flight instrument. This work was jointly supported by this grant and National Institute of Neurological Diseases and Blindness Grant NB-04270, and Air Force Grant AF-AFOSA-886-65, and was submitted to NASA in IRL Report 1035.

problems of UV Microspectrophotometry. These efforts have now been concentrated on the problem of data read-out of the Model E (Beckman) ultracentrifuge. This work was described in detail in a previous progress report and technical report IRL #1014.

Low light intensities necessitate large frame storage for a satisfactory video signal-to-noise. Even a high intensity zenon light source, after filtering to achieve the appropriate spectral distribution, gives light intensities much too low for practical applications of the video signal. For this reason our efforts on this program have continued. A more sensitive camera tube or a brighter light source is required. Because of cost and simplicity we have decided to attempt to improve the light source. The light source presently used (PEK-X-75 zenon arc lamp) is believed to be one of the brightest sources in the band of 2600 Å. However, one can utilize this source to achieve higher intensities by increasing the peak power input in such a manner as to keep the average power input within the lamp specifications. Since the interval of interest is only a small portion of each revolution of the rotor (cell size about 1/1000 of the perimeter), pulsing the lamp with synchrony and increasing the input power by a factor identical to the duty cycle mentioned, an increase in light output is expected.

This method of obtaining high peak light intensity was reported by J.L.Emmett and A.L. Schawlow [Appl. Phys. Letters. Vol. 2, 204 (1963)]. Due to cycle-times of 1 millisecond, however, much lower peak power densities are permitted than those reported.

This approach is presently under investigation and preliminary data indicate that the peak and average light output is increased by increasing the input peak power of the source. Rough experimental results indicate that the output signal of the vidicon camera tube is proportional to the square of the peak current input of the source at the visible spectrum. Since the detector used has a gain factor (μ) close to one, the measured voltage or current is directly proportional to the incident illumination. Therefore, for this particular light source a simplified equation for the transfer function is:

$$\text{Illum} = AI^{E}_{\text{current}}$$

It is found that $E = 2$.

This result compares with those given in the above reference, although the repetition rate was much higher in their particular case. Undoubtedly the transfer function given is strongly dependent on the wavelength. It has been observed that E increases at shorter wavelengths.

This pulsed light system is being installed in the ultra-centrifuge and synchronized with operation of the ultra-centrifuge.

VII. Membrane Permeability

A good deal of our efforts on selective membrane permeability has been reported in previous published reports. A final report on this work has been prepared (IRL #1012). This has shown that the selective properties of membranes can be used to design assays such as for the metabolism of labelled CO_2 from labelled glucose. Such membrane properties could also be used for fluorescent assays such as the one described above for DN'ase. Mr. Lundstrom,

with support from this grant, pursued this work further under the supervision of Professor E. Hutchinson, of the Department of Chemistry. His Master's thesis "A Theory of Molecular Transport Phenomena through Thin Membranes" has been submitted as a report (IRL #1034). No further work in this direction is presently contemplated.

VIII. Biological Applications of Nuclear Magnetic Resonance.

Three published papers [papers 7, 8, (1964) and 23 (1965)] describe the work that was done on the biological applications of nuclear magnetic resonance. No further work in this direction is presently contemplated.

IX. Carbon Metabolism.

Dr. Elie Shneour carried out a study on the oxidation of graphitic carbon to CO_2 in certain soils. He was able to demonstrate a substantially higher rate of oxidation in non-sterile soil than in treated controls. These studies are presented in a paper submitted for publication (IRL#1039).

PERSONNEL AND ORGANIZATION

The present organization and staff of the Instrumentation Research Laboratory is indicated below:

Principal Investigator: Professor J. Lederberg

Cooperating Investigators: Professor Carl Djerassi
Department of Chemistry

Professor Lubert Stryer
Department of Biochemistry

Professor Edward A. Feigenbaum
Department of Computer Science

Professor John McCarthy
Department of Computer Science

Professor Peter Z. Bulkeley
Mechanical Engineering
Director Design Division

Program Director: Dr. E. Levinthal

Biochemistry Group: Dr. J.W. Westley, Dr. Berthold Halpern, and 3 laboratory assistants.

Electrical Engineering Group: William Bonner, Lee Hundley, Walter Reynolds, Nicholas Veizades, and 2 laboratory technicians.

Physics Group: Dr. Sidney Liebes, Dr. George Slodzian (Dr. Slodzian plans to return to the Université de Paris this fall).

Programing Group: Richard K. Moore, Timothy Coburn, Robert Tucker.

In addition, there are part-time programming, and full-time machine shop and office supporting personnel. Mr. Donald Stuedeman joined the laboratory as an administrative assistant this July.

The Following curriculum vitae are for Dr. Liebes, Mr. Reynolds, and Mr. Stuedeman.